

63 EXPERIMENTALLY INDUCED EARLY DEGENERATION OF THE HIP JOINT IN A SHEEP MODEL

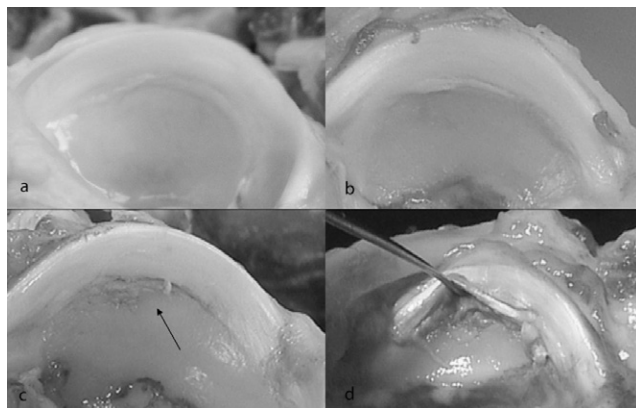
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Purpose: A non-spherical femoral head is a known risk factor for early osteoarthritis of the hip joint. Recently, a new pathomechanism termed femoro-acetabular impingement has been postulated as an underlying cause. This mechanism is postulated to represent an abutment conflict between the non-spherical portion of the head/neck junction and the acetabular rim during extreme flexion and internal rotation. It is thought to lead to repetitive trauma resulting in subsequent lesions of the labrum and adjacent cartilage at the acetabular rim or femoral head. The aim of this research study is to confirm in an animal model, that a non-spherical head contour leads to primary lesions at the acetabulum and at the non-spherical portion of the femoral head similar to the findings in human hip joints.

Methods: Sheep have been chosen as animal model since the acetabular anatomy is close to that in humans including the presence of a labrum and the femoral head has a distinct non-spherical extension of the cartilage in its superior portion. By performing an intertrochanteric adduction osteotomy of the proximal femur the non-spherical portion was rotated toward the weight bearing zone and created similar impingement conditions during the end range of flexion as postulated in young adult human beings. In a pilot study with 6 sheep localized peripheral hip joint lesions could be produced at the acetabular rim and the non-spherical portion of the head similar to the finding in humans. For this study a total of 16 sheep was chosen. The sheep were divided into 4 groups, each group with 4 sheep that were sacrificed after 14, 22, 30, 38-weeks after surgery. After sacrifice the joint cartilage was evaluated for visible alterations macroscopically according to grading by Beck et al. Thereafter samples were harvested of standardized segments on both, the operated and non-operated (control) sides for histological work-up using conventional staining methods with Toluidin-blue O to evaluate proteoglycan depletion and grading of cartilage alterations according to Mankin.

Results: Localized labral lesions as also typically seen in humans were present in 14 out of 16 hips on the operated side versus 2 lesions on the unoperated side. Cartilage lesions including flaps, tears or superficial fraying were seen in all 16 operated hips in the caudodorsal segment of the acetabulum versus alterations in 3 out of 16 hips on the unoperated side (different stages of alteration showed in Figure). Histochemical staining confirmed the macroscopic findings and showed statistically significant higher degenerative changes according to the Mankin staging in the caudodorsal segment on the operated side versus the non-operated side.

Conclusions: With the presented animal model a cam impingement-mechanism and localized, peripheral intraarticular lesions of the hip similar those seen in humans could be reproduced. Therefore a model of development of early osteoarthritis in the hip with different stages of the disease could be defined. This allows to develop and to evaluate new invasive and non-invasive treatment methods for this pathomechanism. It will be also used to further clarify the sequential steps of the osteoarthritic process and help to define the ideal time point for surgical interventions.



Sheep acetabulum control side and different time interval after surgery: normal cartilage control side (a), early surface degeneration ("carpet phenomenon") (b), malacia of cartilage (c) and severe degeneration and labral damage (d).

64 TRANSGLUTAMINASE 2 IS A BIOMARKER OF BOTH STRAIN-SPECIFIC OSTEOARTHRITIS SEVERITY AND CHONDROCYTE HYPERTROPHY IN GUINEA PIG KNEES

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Purpose: The maturation of chondrocytes to hypertrophy is an event that promotes dysregulated matrix repair, calcification, and disease progression in OA. Chondrocyte hypertrophy results in a remodeling of the extracellular matrix, partly through a shift in cartilage-specific collagens from type II to type X collagen, enhanced expression of MMP-13 and ADAMTS-5, and diminished aggrecan expression. Previously, we identified transglutaminase (TG) 2, identified as a modulator of chondrocyte differentiation in OA cartilage, as a novel and TG-family specific biomarker of spontaneous OA in the Hartley guinea pig model. To further explore the role of chondrocyte hypertrophy in the development of OA in this animal model, we examined expression levels of type II and type X collagens, MMP13, and ADAMTS-5 in medial and lateral tibial cartilage in Hartley guinea pigs at specific time points, as well as in an OA-resistant strain, Strain 13.

Methods: Animals: Male Hartley guinea pigs were obtained at two months of age and sacrificed at 2, 4, 7, 10, 12 and 18 months of age (n=6 in each group). Strain 13 guinea pigs were sacrificed at 2 and 12 months of age. One knee joint was prepared for histological grading and cartilage was harvested separately from the medial and lateral compartments of the other knee joint, pooling cartilage from each time point to provide a quantity of tissue sufficient for RNA isolation and subsequent production of cDNA. All aspects of this study were humane and Institutional Animal Care and Use Committee approved. PCR: Real-time qPCR was used to quantify the expression levels of TG2, MMP-13, type II collagen, type X collagen, and ADAMTS-5 from both medial and lateral tibial plateau compartments using the LightCycler (Roche). Statistical analyses: Student's t-test for paired samples was used to assess differences in quantitative mRNA expression levels. A p-value <0.05 was considered significant.

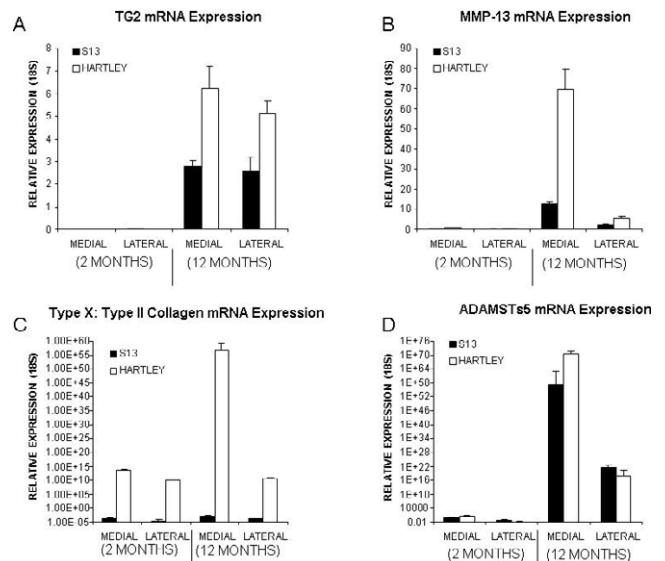


Figure 1.

Results: Steady-state mRNA levels of MMP-13, type X:type II collagen ratio, and ADAMTS-5 increased in the medial compartment of the Hartley guinea pigs at 12 months of age. Comparing Hartley and Strain 13 guinea pigs at selected time points, we observed higher levels of TG2 mRNA in Hartley than Strain 13 guinea pig knees, and particularly so in the medial compartment (Figure 1A, $p = 0.00231$). In addition, mRNA levels of MMP-13 and the type X:type II collagen mRNA ratio were elevated in the medial tibial plateau in Hartley, but not Strain 13 guinea pigs at 12 months of age (Figure 1B, C, $p = 0.0012$ for MMP-13 where indicated, and $p = 0.04$ and $p = 0.0054$ at 2 and 12 months, respectively for the type X:type II collagen mRNA ratio). Interestingly, although levels of ADAMTS-5 mRNA were elevated in the medial compartment at 12 months of age, this expression pattern was not specific to the Hartley strain guinea pigs (Figure 1D).

Conclusions: The knee cartilage of the Hartley guinea pig undergoes an increase in the expression of genes associated with hypertrophy by 12 months of age coincident with the development of severe osteoarthritic changes. Compared to the OA-prone Hartley strain, Strain 13 guinea pig knee cartilage showed much lower expression of genes associated with the hypertrophic cartilage phenotype. These findings are compatible with the lesser severity of OA in this model. Interestingly, Hartley and Strain 13 guinea pigs showed similar and high ADAMTS-5 mRNA expression, suggesting that ADAMTS-5 may be relevant in OA development in the guinea pig model, but may not account for the differential susceptibility of the two strains to OA.

65 FRIZZLED RELATED PROTEIN (FRZB) IS INVOLVED IN DIFFERENT ASPECTS OF JOINT BIOLOGY AND PATHOLOGY

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Purpose: The Frizzled related protein (*FRZB*) gene has been associated with susceptibility to osteoarthritis in different cohorts. *FRZB* is a secreted antagonist of the WNT signaling pathway originally identified in a chondrogenic extract of articular cartilage. We have generated *Frzb*^{-/-} mice and demonstrated that this loss of function model shows increased cartilage damage in different induced mouse models of osteoarthritis. Deletion of *Frzb* is associated with increased Wnt signaling and increased matrix metalloproteinase activity and expression. In addition, cortical bone thickness and adaptive bone formation upon loading are increased in *Frzb*^{-/-} mice as compared to wild-types. Here, we further evaluated the spontaneous development of knee and hip osteoarthritis in a cohort of normal aging mice as well as a cohort of mice exposed to voluntary running activity. In addition we studied the effect of *Frzb* deletion on enthesophyte/osteophyte formation in the spontaneous model of arthritis in DBA/1 mice.

Methods: Aging *Frzb*^{-/-} and wild-type mice were evaluated by X-rays at week 26 and 52. In another cohort, *Frzb*^{-/-} and wild-type mice were grouped and a running wheel was introduced into the cage at the age of 7 weeks. At week 8, all mice were caged solitarily with a running wheel available. Mice were allowed free exercise for 6 to 12 months and distances run were recorded daily. At the end of the experiment, mice were sacrificed and joints were processed for histological evaluation. Cartilage damage, synovitis and osteophyte formation were scored. Muscle fiber composition of the soleus and extensor digitorum longus were studied by immunofluorescence. *Frzb*^{-/-} mice were backcrossed onto the DBA/1 background for over 7 generations. Male DBA/1 mice from different litters were caged together at the age of 10 weeks (>5 males per cages) and observed for clinical signs of arthritis. Alternatively wildtype DBA/1 males were treated with intramuscular injections of plasmid containing *Frzb* under control of a CMV promoter every three weeks (week 11, 14, 17 and 20). At the end of these experiments (week 30 and 25 respectively), mice were sacrificed and histological severity of disease was evaluated.

Results: No statistical differences in spontaneous incidence or severity of osteoarthritis were seen between *Frzb*^{-/-} mice and wild-type mice. At the age of 6 months, both female and male wild-type mice showed significantly greater voluntary running activity than the *Frzb*^{-/-} mice ($p < 0.05$). At one year, the difference was still significant for male mice, but not for females. Running exercise did not significantly affect severity of osteoarthritis. Differences in distances run were not explained by abnormalities in muscle composition.

In the spontaneous model of joint remodeling, average clinical disease severity score and average clinical severity score per affect animal were higher in wildtype as compared to *Frzb*^{-/-} mice. Average histomorphological severity score was also higher in wild-type as compared to *Frzb*^{-/-} mice. In contrast, overexpression of *Frzb* by intramuscular plasmid injection did not affect clinical incidence, severity and histomorphological disease severity scores.

Conclusions: Absence of *Frzb* in mice does not lead to increased incidence or severity of osteoarthritis. However, reduced voluntary physical activity in running wheels suggests that absence of *Frzb* affects locomotor function. The underlying mechanisms are not yet fully understood. Deletion of *Frzb* also reduces endochondral bone formation in a mouse model of ankylosis. These combined data further establish a role of *FRZB* and WNT signaling in postnatal musculoskeletal biology and disease. Functional polymorphisms in *FRZB* in humans may also affect osteophyte formation and disease progression

66 ORAL CALCITONIN REDUCES CARTILAGE EROSION IN AN OA RAT MODEL WITH BOTH TRAUMATIC INJURY AND INCREASED SUBCHONDRAL BONE TURNOVER

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Purpose: Osteoarthritis (OA) is a disease that affects the entire articular joint, with both changes to the bone and cartilage compartments. Increased bone turnover, as caused by oestrogen deficiency, has been speculated to augment the severity of cartilage erosion and to accelerate the pathogenesis of OA. An optimal intervention strategy for OA may therefore ideally target both bone and cartilage mal metabolisms. Calcitonin has been shown to affect both osteoclasts and chondrocytes. We investigated whether a novel oral formulation of salmon calcitonin could reduce cartilage erosion and attenuate osteophyte formation, observed secondary to induction of OA by combined partial medial meniscectomy and ovariectomy of rats.

Methods: Four groups of 6 months old rats were subjected to sham, ovariectomy (OVX) or a combined ovariectomy and partial medial meniscectomy (OVX+MNX) and administered twice daily with oral salmon calcitonin (CT) (150 mg/kg 5-CNAC + 2 mg/kg calcitonin) or vehicle control (V) (150 mg/kg 5-CNAC) in the following way: (1) Sham+V; (2) OVX+V; (3) OVX+MNX+V; (4) OVX+MNX+CT. Serum samples were collected at baseline and 3, 6 and 8 weeks after surgery, and weights were recorded at regular intervals. Animals were sacrificed 8 weeks after surgery, and the tibia was processed for histology. C-terminal telopeptide of type II collagen (CTX-II) was measured in the serum. Histological sections were stained with Fast Green/Safranin O. The tibial plateau was equally divided into a medial, central and lateral compartment, subsequently analysed separately where the area of articular cartilage was measured using automatic delineations in ImagePro. Areas of osteophytes situated in the lateral compartment were scored separately.

Results: OVX surgery increased serum CTX-II levels by 260%, 240% and 53% in week 3, 6 and 8 after surgery, respectively. Meniscectomy did not further increase the systemic CTX-II levels. Treatment with oral calcitonin significantly ($p < 0.05$) reduced CTX-II concentrations to below sham levels. Ovariectomy alone resulted in a moderate cartilage loss of 5.8% in the central tibial compartment compared to sham animals, whereas OVX+MNX+V caused a heavy 33.3% ($p < 0.05$) cartilage loss. Treatment with oral calcitonin reduced that cartilage loss by 52% ($p = 0.06$). The area of laterally occurring osteophytes increased 15-fold ($p < 0.01$) from OVX+V to OVX+MNX+V animals. The effects of oral calcitonin reduced the area of osteophytes by 55% when comparing the group OVX+MNX+V to OVX+MNX+CT.

Conclusions: Currently there are no treatments available for OA. These data are the first to demonstrate that an oral formulation of calcitonin protects against cartilage erosion and osteophyte formation in an *in vivo* OA model with both traumatic injury and increased subchondral bone turnover. The chondroprotective effects of salmon calcitonin may be a combination of direct effect on chondrocytes in addition to the well-established effect on bone resorption. Further clinical studies are needed to validate the herein documented effects on cartilage erosion.

67 DELETION OF ESTROGEN RECEPTORS INDUCES OSTEOPHYTES BUT DOES NOT PROMOTE EARLY CARTILAGE DAMAGE IN MICE

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Purpose: Since the prevalence of osteoarthritis OA is much higher in postmenopausal than in premenopausal women, and cartilage damage is increased after ovariectomy in several animal models, it is believed that estrogen depletion plays a role in the onset or progression of OA. Estrogen acts via the estrogen receptors (ERs) α and β . It is known that deletion of ERs leads to changes in the metaphyseal bone. We hypothesize that deletion of one or both estrogen receptors in female mice will promote osteoarthritic changes in the cartilage and subchondral bone.

Our aim was to study cartilage and subchondral bone changes in knee joints of female ER α -/-, ER β -/-, and double ER α -/-ER β -/- mice.

Methods: Tibias of 6-month-old female ER α -/-, ER β -/-, and ER α -/-ER β -/- mice and their wildtype (wt, C57Bl/6 background) littermates ($n \geq 6$ per group) were analyzed with microCT and histology.